

**R E M A R K S**

**Status of the Application**

Claims 1-8, 10-22, 24-28, 30, 31, and 33-36 are pending in the present application.

Claims 13, 27, 30, 31, 33, and 34 have been cancelled and Claims 1, 15, 35, and 36 have been amended, notwithstanding Applicants' belief that the cancelled and amended claims would have been allowable, without acquiescing to any of the Examiner's arguments, and without waiving the right to prosecute the unamended (or similar) claims in another application, for the purpose of furthering Applicants' business goals and expediting the patent application process in a manner consistent with the PTO's Patent Business Goals (PBG).<sup>1</sup> None of the amendments to the claims is related to the statutory requirements of patentability unless expressly stated so herein. No amendment made herein was intended to narrow the scope of any of the amended claims within the meaning of *Festo*.<sup>2</sup>

In particular, Claims 13 and 27 have been cancelled to avoid lack of antecedent basis for the term "mouse embryonic stem cell".

Claims 30, 31, 33, and 34 have been cancelled in favor of newly added Claims 37-50 which recite using mouse embryonic stem cells.

Claims 1, 15, 35, and 36 have been amended by deleting the recitation of "mouse embryonic stem cells." This amendment is not a narrowing amendment within the meaning of *Festo*, since the cancelled subject matter is presented in newly added Claims 37-50.

New Claims 37-50 have been added to describe preferred embodiments of the invention. More specifically, new Claim 37 recites treating mouse embryonic stem cells with a chemical agent under conditions such that "at least one modification is produced in substantially every gene in said mouse embryonic stem cells." Support for this recitation is

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<sup>1</sup> 65 Fed. Reg. 54603 (September 8, 2000).

<sup>2</sup> *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.*, No. 95-1066, 2000 WL 1753646 (Fed. Cir. Nov. 29, 2000).

found in the Specification on, for example, page 13, lines 26-28,<sup>3</sup> page 14, lines 3-6,<sup>4</sup> page 15, lines 24-26,<sup>5</sup> and page 18, lines 10-13.<sup>6</sup>

New Claims 38, 39, and 40 recite that at least one modification in at least 70%, 85%, and 95% (respectively) of the genes in the mouse embryonic stem cells is produced. This is supported by the Specification's disclosure that "The term 'substantially every gene' refers to the statistical probability, preferably at least about 70% probability, more preferably at least about 85% probability, and most preferably at least about 95% probability."<sup>7</sup>

New Claim 41 recites that the "number of said isolated mouse embryonic stem cells in said *in vitro* culture consists of from 200 to 600 cells." This finds support in the Specification which discloses "that at least one point mutation may be introduced into every gene by treating as few as from about 200 to about 600 cells with ENU."<sup>8</sup>

New Claims 42 and 48 recite the step of using treated mouse embryonic stem cells to generate a mouse, as supported by the Specification's disclosure of methods for generating transgenic mice using mouse embryonic stem cells.<sup>9</sup>

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<sup>3</sup> The Specification states that "The methods of the invention also provide a set of cells which contain one or more modifications in **substantially every gene** within the genome of the cells (*i.e.*, the "Library"). (Emphasis added).

<sup>4</sup> The Specification recites that "The methods of the invention involve treating a population of cells with one or more agents (*e.g.*, nucleic acid sequence-modifying agents) which are capable of introducing one or more modifications in **substantially every gene** within the genome of the cells." (Emphasis added).

<sup>5</sup> The Specification states that "The modified cells which collectively harbor an allelic series of modifications in **substantially every gene** are particularly useful in investigating diseases which are associated with more than one modification in a given gene." (Emphasis added)

<sup>6</sup> The Specification states that "Treatment with the nucleic acid sequence-modifying agent is contemplated to produce the Library, *i.e.*, a set of cells which collectively contain one or more modifications in **substantially every gene** within the genome of the cells." (Emphasis added).

<sup>7</sup> Specification, page 14, lines 6-10.

<sup>8</sup> Specification, page 23, lines 15-18.

<sup>9</sup> Specification, page 28, lines 3-12.

New Claims 43, 44, 49, and 50 which recite that the gene of interest is associated with a disease are supported by the Specification's disclosure of the recited disease-associated genes.<sup>10</sup>

New Claims 45 and 47 recite the additional step of "detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch." This recitation is supported by the Specification's disclosure that genomic DNA may be screened for mutations using fluorescent chemical cleavage of mismatch (FCCM).<sup>11</sup>

New Claim 46 recites treating isolated mouse embryonic stem cells with *N*-ethyl-*N*-nitrosourea "under conditions such that the frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9,000." This recitation finds support in the Specification's disclosure that "ENU induces mutations in the *Hprt* gene at a frequency of from about 1/600 to about 1/9,000."<sup>12</sup>

These amendments do not introduce new matter.

In the instant Office Action, the Examiner:

1. required an oath or declaration by the actual inventors;
2. rejected Claims 1-8, 10-22, 24-28, 30, 31, and 33-36 under 35 U.S.C. §102(e) over Schafer *et al.* (U.S. Patent No. 6,033,861); and
3. rejected Claims 1-8, 10-22, 24-28, 30, 31, and 33-36 under 35 U.S.C. §102(e) over Goodfellow (U.S. Patent No. 6,015,670).

Applicants believe that the present amendments and the following remarks addresses the Examiner's requirements and traverse the Examiner's rejection of the claims. These remarks are presented in the same order as they appear above.

#### **1. Substitute Declarations Are Submitted**

The Examiner found that "Applicants' Petition for correction of the inventorship filed April 24, 200 is deficient because: An oath or declaration by each actual inventor or inventors

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<sup>10</sup> Specification, paragraph bridging pages 15-16.

<sup>11</sup> Specification, page 38, lines 9-11.

<sup>12</sup> Specification, page 35, lines 4-5.

listing the entire inventive entity has not been submitted.<sup>13</sup> Applicants hereby submit executed substitute Declarations which list the names of the four co-inventors in a single document, thereby overcoming the alleged deficiency of the prior Declaration.

**2. Rejection of Claims 1-8, 10-22, 24-28, 30, 31, And 33-36 Under 35 U.S.C. §102(e) over Schafer *et al.* (U.S. Patent No. 6,033,861)**

The Examiner rejected Claims 1-8, 10-22, 24-28, 30, 31, and 33-36 under 35 U.S.C. §102(e) over Schafer *et al.*<sup>14</sup> (U.S. Patent No. 6,033,861).<sup>15</sup> Applicants respectfully traverse since Schafer *et al.* does not disclose the recited step of "isolating" cells that contain a modified gene of interest. The law is settled that:

"Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration."<sup>16</sup> "[A]bsence from the reference of any claimed element negates anticipation."<sup>17</sup>

Schafer *et al.* discloses using chemical mutagens<sup>18</sup> to mutagenize whole organisms or a selected tissue of an organism, including mutagenesis of germline cells of an organism, such as sperm stem cells, or ova, mutagenesis of embryonic stem (ES) cells of an organism. Following DNA analysis of a specific tissue for a mutation in a gene of interest, such as mutated ES clones in culture, the cells are transferred to the developing embryo.<sup>19</sup>

It is notable that Schafer *et al.*'s treatment of the cells with a chemical results in the production of a mixture of cells containing a modified and unmodified gene of interest, and that this **mixture of cells** is used **directly** to generate mutagenized animals, **without** the prior

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<sup>13</sup> Office Action, page 2, second paragraph.

<sup>14</sup> Schafer *et al.* was filed on November 18, 1998, and issued on March 7, 2000.

<sup>15</sup> Office Action, page 3.

<sup>16</sup> *W.L. Gore & Assoc., Inc v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303, 313 (Fed. Cir. 1983), cert. denied, 105 S. Ct. 172 (1984), citing *Soundscriber Corp. v. U.S.*, 360 F.2d 954, 960, 148 USPQ 298, 301, adopted, 149 USPQ 640 (Ct. Cl. 1966).

<sup>17</sup> *Rowe v. Dror*, 42 USPQ2d 1550, 1553 (Fed. Cir. 1997), citing *Kloster Speedsteel AB v. Crucible, Inc.*, 793 F.2d 1565, 1571, 230 USPQ 81, 84 (Fed. Cir. 1986).

<sup>18</sup> Schafer *et al.*, column 10.

<sup>19</sup> Schafer *et al.*, paragraph bridging column 6 and 7.

isolation of cells that contain the modified gene of interest. Thus, the step of "isolating" cells having a modified gene of interest is absent from Schafer *et al.* This negates anticipation. Accordingly, the rejection of Claims 1-8, 10-22, 24-28, 30, 31, and 33-36 under 35 U.S.C. §102(e) over Schafer *et al.* should be withdrawn.<sup>20</sup>

3. **Rejection of Claims 1-8, 10-22, 24-28, 30, 31, And 33-36 Under 35 U.S.C. §102(e) over Goodfellow (U.S. Patent No. 6,015,670)**

The Examiner rejected Claims 1-8, 10-22, 24-28, 30, 31, and 33-36 under 35 U.S.C. §102(e) over Goodfellow<sup>21</sup> (U.S. Patent No. 6,015,670).<sup>22</sup> Applicants respectfully disagree because Goodfellow does not disclose all the limitations of the claims.

The Examiner argued that Goodfellow discloses "methods of chemical mutagenesis of *embryonic stem cells* for identifying a mutation in a gene of interest in an organism . . . [and] that ENU, MNU, procarbazine hydrochloride, and chlorambucil are especially useful for mutagenesis of *male germ cells*".<sup>23</sup> However, the cell type which is recited in the rejected claims as amended herein is **different** from the cell type which is manipulated by Goodfellow; more specifically, the instant claims recite "embryonic cells selected from the group consisting of fertilized egg cells and cells of 2-cell embryos." Since Goodfellow's embryonic stem cells and male germ cells are not recited in the claims, Goodfellow cannot anticipate. Accordingly, the rejection of the claims under 35 U.S.C. §102(e) over Goodfellow should be withdrawn.

Further, with respect to the newly added Claims 37-50, Applicants note that Goodfellow does not anticipate these claims since it does not disclose Claims 37-45's recitation of treating mouse embryonic stem cells with a chemical agent under conditions such

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<sup>20</sup> Applicants note that newly added Claims 37-50 are also **not** anticipated by Schafer *et al.* since each of the new claims recites "isolating" cells having a modified gene of interest.

<sup>21</sup> Goodfellow issued on January 18, 2000 from a patent application which was filed on May 16, 1997, and which claims priority to a provisional patent application filed on May 17, 1996.

<sup>22</sup> Office Action, page 3.

<sup>23</sup> (Emphasis added) Office Action, paragraph bridging pages 3 and 4.

that "at least one modification is produced in substantially every gene in said mouse embryonic stem cells," and Claims 46-50's recitation of treating isolated mouse embryonic stem cells with *N*-ethyl-*N*-nitrosourea "under conditions such that the frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9,000."

**Conclusion**

All grounds of rejection and objection of the Office Action of March 15, 2001 having been addressed, reconsideration of the application is respectfully requested. It is respectfully submitted that the invention as claimed fully meets all requirements and that the claims are worthy of allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourage the Examiner to call the undersigned collect at (617) 252-3353.

Dated: September 12, 2001



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**APPENDIX I**

**MARKED-UP VERSION OF REWRITTEN, ADDED, AND/OR CANCELLED CLAIMS**

The following is a marked-up version of the claims pursuant to 37 C.F.R. §1.121 (c)(1)(ii) with instructions and markings showing changes made herein to the previous version of record of the specification and claims. Brackets denote deleted text, and underlining denotes added text.

**IN THE CLAIMS:**

Cancel Claims 13, 27, 30, 31, 33, and 34.

Amend Claims 1, 15, 35, and 36, and add new Claims 37-50 as follows:

1. (Four times amended) A method of producing a modification in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells[,] and cells of 2-cell embryos[, and mouse embryonic stem cells];

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells comprising said gene of interest is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest and embryonic cells having a modified gene of interest; and

c) isolating said embryonic cells having a modified gene of interest.

15. (Four times amended) A method of producing an allelic series of modifications in a gene of interest contained in a cell, comprising:

a) providing:

- i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells[,] and cells of 2-cell embryos[, and mouse embryonic stem cells];
  - ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;
- b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest, embryonic cells having a first modification in said gene of interest, and embryonic cells having a second modification in said gene of interest; and
- c) isolating said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic cells.

35. (Twice amended) A method of producing a modification in a gene of interest in a cell, comprising:

- a) providing:
  - i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells[,] and cells of 2-cell embryos[, and mouse embryonic stem cells];
  - ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;
- b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells comprising said gene of interest is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest and embryonic cells having a modified gene of interest;
- c) isolating said embryonic cells having a modified gene of interest; and

d) placing at least one of said embryonic cells having a modified gene of interest into an environment under conditions so as to generate a non-human animal comprising said modified gene of interest.

36. (Twice amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells[,] and cells of 2-cell embryos[, and mouse embryonic stem cells];

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells comprising said gene of interest is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest, embryonic cells having a first modification in said gene of interest, and embryonic cells having a second modification in said gene of interest;

c) isolating said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic cells; and

d) placing at least one embryonic cell selected from the group consisting of said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest into an environment under conditions so as to generate a non-human animal comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

37. (New) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

- i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;
  - ii) a chemical agent capable of producing at least one modification in said gene of interest;
- b) treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and (ii) a mixture of embryonic stem cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest; and
- c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

38. (New) The method of Claim 37, wherein said treating is under conditions such that at least one modification in at least 70% of the genes in said mouse embryonic stem cells is produced.

39. (New) The method of Claim 37, wherein said treating is under conditions such that at least one modification in at least 85% of the genes in said mouse embryonic stem cells is produced.

40. (New) The method of Claim 37, wherein said treating is under conditions such that at least one modification in at least 95% of the genes in said mouse embryonic stem cells is produced.

41. (New) The method of Claim 37, wherein the number of said isolated mouse embryonic stem cells in said *in vitro* culture consists of from 200 to 600 embryonic stem cells, and said chemical agent is *N*-ethyl-*N*-nitrosourea.

42. (New) The method of Claim 37, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

43. (New) The method of Claim 37, wherein said gene of interest is associated with a disease.

44. (New) The method of Claim 43, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.

45. (New) The method of Claim 37, further comprising step d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.

46. (New) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

- a) providing:
  - i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;
  - ii) *N*-ethyl-*N*-nitrosourea;

- b) treating said mouse embryonic stem cells with said *N*-ethyl-*N*-nitrosourea to produce treated mouse embryonic stem cells comprising a mixture of embryonic stem cells, said mixture comprising embryonic stem cells having a first

modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest, wherein the treatment is under conditions such that the frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9,000; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

47. (New) The method of Claim 46, further comprising step d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.

48. (New) The method of Claim 46, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

49. (New) The method of Claim 46, wherein said gene of interest is associated with a disease.

50. (New) The method of Claim 49, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.